

Influence of immunosuppression on the pharmacokinetics and pharmacodynamics of azithromycin in infected mouse tissues

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Azithromycin has been shown to preferentially distribute to infection loci. Due to the potential contribution of phagocytes as transporters of drug to these sites, there has been some concern that immunosuppression of the cellular arm of the host defence system would greatly reduce the delivery of azithromycin to sites of infection and hence impair efficacy. Therefore, we evaluated the pharmacokinetics and pharmacodynamics of azithromycin in a *Staphylococcus aureus* intramuscular infection model in normal and immunosuppressed mice, employing therapeutic and prophylactic regimens. Immunosuppression was induced by daily doses of cyclophosphamide that culminated in leucopenia with an underlying granulocytopenic condition, with circulating peripheral granulocytes numbering from $\leq 0.1\text{--}0.3 \times 10^9/\text{L}$. Azithromycin tissue levels were not reduced in infection loci in granulocytopenic mice but moderate increases in C_{\max} and AUC values were observed, relative to similar tissues from normal mice. The tissue half-life of azithromycin in infected tissues in a therapeutic mode (75 h) was three-fold longer than in a prophylactic mode (25 h); this correlated with the degree of inflammation (therapy was withheld until inflammation was evident; i.e., prophylaxis reduced inflammation). Histological examination of infected tissues from normal and leucopenic mice was indistinguishable despite a 70%–85% reduction in circulating granulocytes. Compared with untreated infected controls, bactericidal activity was noted following prophylaxis with azithromycin and bacteraemia was suppressed in mice receiving azithromycin therapeutically. In summary, these data indicate that azithromycin delivery and efficacy in a moderately immunosuppressed animal model are unimpaired.

Introduction

Azithromycin, the first azalide antimicrobial agent (Bright *et al.*, 1988), achieves high tissue concentrations, which greatly exceed the corresponding serum concentrations. The persistently high tissue levels of azithromycin are a consequence of low serum binding and an extremely large distribution volume which results from rapid and extensive concentration of drug within the intracellular and interstitial compartments of tissues (Foulds, Shepard & Johnson, 1990; Schentag & Ballow, 1991). The pharmacokinetics of azithromycin suggest that tissue levels are the most relevant predictor of efficacy, and this has been confirmed by the successful therapy of infections of skin/soft tissues, the respiratory tract and sexually transmitted diseases (Lassus, 1990; Steingrimsson *et al.*, 1990).

Azithromycin is rapidly taken up by phagocytes, concentrates up to 300-fold the extracellular concentration, and is slowly released following the decline in extracellular concentrations (Gladue *et al.*, 1989; Laufen, Wildfeuer & Lach, 1990). Recent studies have demonstrated a correlation between the increased levels of azithromycin found within infection sites and phagocytic infiltration (Girard D. *et al.*, 1990; Retsema *et al.*, 1993). These observations suggest that the uptake, accumulation, transport and deposition of azithromycin by phagocytes at sites of infection may be an ancillary mechanism of drug distribution and delivery to these infection sites.

The immunosuppressed state, specifically granulocytopenia, is a compromised condition that results in decreased resistance to infection. In granulocytopenia the number of peripheral blood granulocytes is reduced; this reduction can be induced by various disease states or through the use of cytotoxic drugs (Hartlapp, 1987). Since a phagocytic delivery mechanism has been suggested for azithromycin, we evaluated the pharmacokinetics and pharmacodynamics of azithromycin in infected tissues under conditions of leucopenia with an underlying granulocytopenia and intact immunity.

Materials and methods

Pharmacological agents

Azithromycin was supplied by the Central Research Division, Pfizer Inc, Groton, CT, USA. Cyclophosphamide was obtained from Sigma Chemical Co., St Louis, MO, USA.

Animals

Male outbred CD-1 mice (24–26 g) were obtained from Charles River Breeding Laboratories, Kingston, NY, USA. All procedures conducted in mice were in accordance with accepted Institutional Animal Care and Use Committee guidelines and when necessary euthanasia was accomplished by CO₂ asphyxiation.

Bacterial strain

A mouse-passaged strain of *Staphylococcus aureus*, Pfizer culture designation 01A052, (clinically derived β -haemolytic strain that is penicillin- and macrolide-susceptible, azithromycin MIC/MBC: 0.4/6.3 mg/L) was used in an intramuscular infection model in mice.

Therapeutic model

In the therapeutic model, CD-1 mice were divided into normal (intact immunity) and immunosuppressed groups. Immunosuppression was induced by daily doses of cyclophosphamide (50 mg/kg, ip) beginning 3 days before infection and continuing until the day before termination of the study (the body weight of cyclophosphamide-treated mice was approximately 25% less than the body weight of normal mice at the termination of the study). A suspension of washed *S. aureus* cells ($\sim 10^6$ cfu per 0.1 mL) was injected into the left caudal thigh of normal and immunosuppressed mice, and the bacterial population was allowed to proliferate for 18 h before azithromycin therapy

(200 mg/kg, po). Contralateral caudal thighs (left and right thighs from the same animal) were aseptically excised from killed mice following exsanguination (ten mice per time point) at 0, 2, 5, 24, 48, 72 and 96 h following azithromycin administration. The thighs were subsequently weighed, diluted with 2.5 mL 0.05 M phosphate-buffered saline (PBS, pH 6.0) and homogenized using a Tissue Mizer apparatus with attached 10 × 100 mm generator probe (Tekmar Co., Cincinnati, OH, USA).

Prophylactic model

Immunosuppression was induced by daily doses of cyclophosphamide (20 mg/kg, ip) beginning 4 days before infection and continuing until the day before termination of the study (the mean body weight of cyclophosphamide-treated mice was ~10% less than the mean body weight of normal mice at the termination of the study). A suspension of washed *S. aureus* cells (~10⁶ cfu per 0.1 mL) was injected into the left caudal thigh of control and immunosuppressed mice. Azithromycin was administered orally to mice (100 mg/kg) 1 h following bacterial challenge. Thigh samples were obtained following exsanguination (ten mice per time point) at 0, 2, 4, 24, 48, 72, 120 and 144 h following azithromycin administration and processed as in the therapeutic model.

Quantitation of white blood cells

Before the mice were killed, peripheral blood samples were taken from the orbital sinus venous plexus of normal infected and immunosuppressed infected mice and collected in Microvette CB 100 sodium EDTA-coated vessels (Sarstedt, Numbrecht, Germany). Blood samples from control CD-1 mice (non-infected and non-immunosuppressed) at each sampling time were obtained to establish the normal baseline WBC count. Total WBC counts were performed using an ELT8-DS automated cell counter (Becton Dickinson and Co., Sunnyvale, CA, USA). Differential counts were performed following methylene blue staining by a Hematrack automated cell differentiation instrument (Beckman Instruments Inc, Palo Alto, CA, USA).

Bacteraemia evaluation

Blood samples were taken from non-infected control mice (negative control), infected control mice (positive control) and from infected mice from the therapeutic model at 48 h after azithromycin administration. Ten μ L of each blood sample was diluted into a 100 μ L volume of PBS (pH 7.4), dispensed on to the surface of a sheep blood agar plate and uniformly distributed over the agar surface with the use of a glass rod. The spread plates were incubated at 37°C in air for 24 h, and mice were considered bacteraemic if any β -haemolytic cfu appeared on the agar surface (> 100 cfu per mL).

Enumeration of colony forming units from thigh tissues

After homogenization of the tissue samples, aliquots from each sample were taken and serial ten-fold dilutions were prepared in PBS and dispensed on to BHI agar plates. Parallel control samples were run by fortifying PBS and thigh tissue homogenates from

control mice with *S. aureus* and proceeding as above in order to evaluate recovery of organism from the tissues (recovery was complete). The agar plates were incubated aerobically at 37°C for 18 h, *S. aureus* cfu counted, and cfu were reported as the geometric mean per g of thigh tissue.

Bioassay for azithromycin

Azithromycin drug concentrations were determined by an agar well bioassay using *Micrococcus luteus* (ATCC 9341) as the assay organism (Girard *et al.*, 1987). The tissue homogenates (following removal of aliquots for cfu determination) were centrifuged at 16,000 g for 5 min. The supernates were decanted and analysed for azithromycin. Azithromycin standard curves were prepared by fortifying homogenates of control thigh tissues, obtained from non-dosed mice, with a range of azithromycin concentrations and processed as above. Quantitation of azithromycin unknowns was achieved by comparison of zone size against curves prepared by least-squares regression analysis of log concentration versus zone size of the azithromycin standards. The azithromycin thigh tissue concentrations were not corrected for blood-derived azithromycin, since the thigh tissue blood contamination was kept to a minimum due to the prior exsanguination of the mice; the blood-derived contribution was found to account for <1%.

Pharmacokinetic analysis

All results are expressed as the mean and standard error of the mean. The pharmacokinetic parameters were defined as follows. C_{max} was the highest observed thigh tissue concentration. Half-life or $T_{1/2} = \ln 2/k_{el}$; where k_{el} (elimination rate constant) was the slope obtained from least-squares regression analysis of apparently linear portions of the log concentration versus time curve. AUC, the area under the concentration versus time curve from zero to infinity was calculated by the trapezoidal method through the last time point plus the terminal area as determined from the ratio of the last concentration measured to the elimination rate constant (Gibaldi & Perrier, 1982).

Statistical analysis

Four experimental measurements were subjected to analysis of variance (ANOVA): paired difference within the same animal between the infected and non-infected thigh, in tissue weight and in azithromycin concentration; the severity of infection in the affected thigh (*S. aureus* log cfu/g tissue); and the WBC count. The ANOVA was two-way with interaction; the two factors were cyclophosphamide dosing, and hours following azithromycin dose. *P*-values were calculated only for contrasts planned before the experiment. Each analysis was carried out independently for each of the measures, on data from either prophylactic or therapeutic mode of treatment. In addition to the ANOVA, *P*-values were computed from parametric confidence bounds to determine whether the paired differences between infected and non-infected tissues were significantly different from zero, at each post-azithromycin time, only in animals not treated with cyclophosphamide. Appropriate checks were run to assure approximate normality of

distributions and homogeneity of variance of all data subjected to parametric analysis. Overall significance in the ANOVA was required before specific contrasts were computed.

Histology of infected thighs

Time-matched non-infected control and *S. aureus*-infected thighs from normal and immunosuppressed mice were excised and fixed in 10% neutral buffered formalin, dehydrated in a graded series of alcohols, trimmed in a plane perpendicular to the tibia, embedded in paraffin, sectioned at 6 μm with a microtome, mounted on glass slides and stained with haematoxylin and eosin. The slides were examined microscopically and representative photomicrographs were taken at a magnification of $\times 625$.

Results

Therapeutic mode of administration

Peripheral WBC counts were determined to provide evidence of quantitative immunosuppression, as well as to ensure sufficient underlying immunity to avoid lethality due to septicaemia (Figure 1). The baseline WBC count for this strain of mouse (CD-1) was found to be approximately $6 \times 10^9/\text{L}$ (s.e.m. of $0.2 \times 10^9/\text{L}$ WBC) and differential analysis indicated that normal peripheral blood contained $\sim 90\%$ lymphocytes and $\sim 10\%$ granulocytes. Daily doses of cyclophosphamide (50 mg/kg, ip) reduced the peripheral WBC count by $\sim 85\%$ prior to infection ($P < 0.001$) and differential WBC analysis indicated an underlying granulocytopenia ($< 0.1 \times 10^9/\text{L}$ granulocytes). General leucocytosis was evident in normal infected mice and differential analysis confirmed the presence of granulocytosis with a shift in the differential proportion of granulocytes from 5–10% to 20–30%. The underlying granulocytosis in normal infected mice was reduced following azithromycin therapy from $3 \times 10^9/\text{L}$

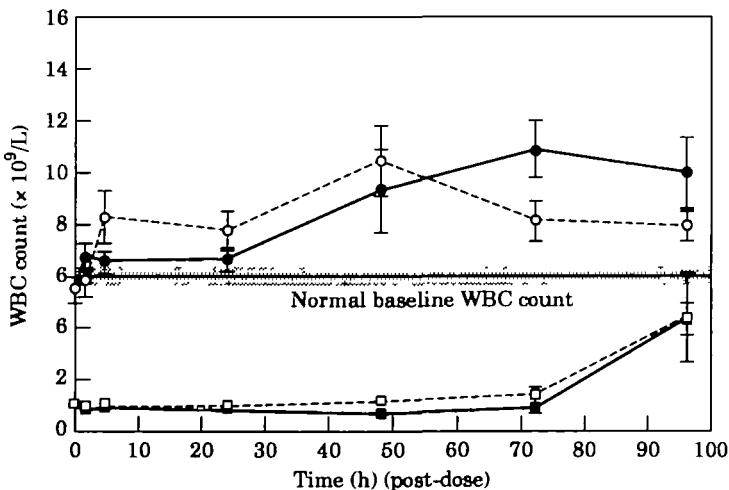


Figure 1. Peripheral WBC count (mean \pm s.e.m.) in *S. aureus*-infected mice. Open symbols represent normal (○) and immunosuppressed (□) mice that received a 200 mg/kg oral dose of azithromycin (therapeutic model). Closed symbols represent normal (●) and immunosuppressed (■) control mice (no azithromycin). The normal baseline WBC count (mean \pm s.e.m.) is depicted in the shaded section.

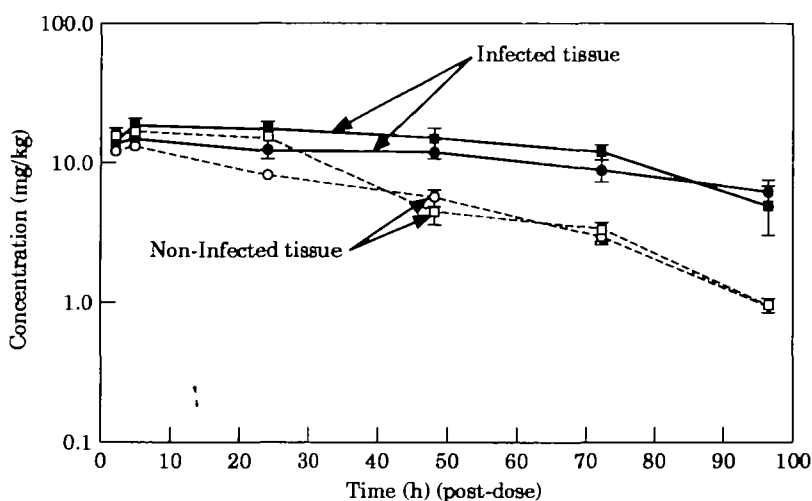


Figure 2. Azithromycin concentrations (mean \pm S.E.M.) in thigh tissues of mice following a 200 mg/kg oral dose (therapeutic model). Closed symbols represent *S. aureus*-infected thigh tissues from normal (●) and immunosuppressed (■) mice. Open symbols represent non-infected thigh tissues from normal (○) and immunosuppressed (□) mice.

granulocytes (S.E.M. of 0.4) to $0.9 \times 10^9/L$ granulocytes (S.E.M. of 0.8) by 120 h post-challenge.

Therapy with azithromycin was delayed for 18 h following *S. aureus* im challenge in normal and leucopenic mice to allow proliferation of the challenge organism and maximum inflammation at the infection site. The observation of Retsema *et al.* (1993) was confirmed during the analyses; i.e. that the inflammation induced by the bacterial infection was associated with an increase in the mean thigh tissue weights relative to the non-infected thigh tissue weights in both normal and leucopenic mice ($P < 0.01$). Azithromycin concentrations in *S. aureus*-infected and non-infected thigh tissues from normal and leucopenic mice following administration of a 200 mg/kg oral dose are presented in Figure 2. The infected and non-infected thigh tissue C_{max} values for azithromycin from leucopenic mice were essentially equivalent, as were the corresponding values from normal mice (Table I). Although the C_{max} for infected and non-infected thigh tissues from leucopenic mice were slightly higher than the corresponding values from normal mice, the difference was not significant. The elimination half-life of azithromycin in infected thigh tissue was about three-fold longer

Table I. Mean (S.E.M.) pharmacokinetic parameters of azithromycin in *S. aureus*-infected and non-infected mouse thigh tissues following administration of a 200 mg/kg oral dose given in a therapeutic mode

Parameter	Thigh tissues type			
	normal infected	normal non-infected	leucopenic infected	leucopenic non-infected
C_{max} (mg/kg)	18.1 (1.4)	15.0 (1.1)	22.8 (1.9)	20.9 (0.8)
$T_{1/2}$ (h)	76.7 (19.9)	27.9 (2.7)	71.2 (15.0)	23.4 (2.5)
$AUC_{0-\infty}$ (mg · h/kg)	1413 (89)	646 (26)	2153 (299)	810 (61)

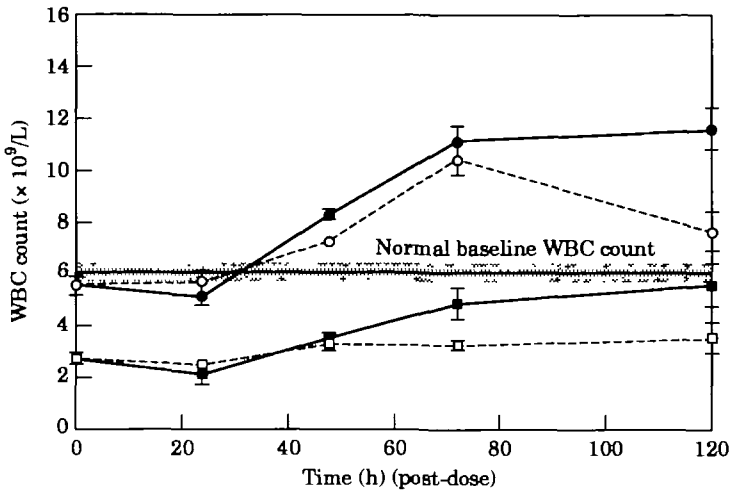


Figure 3. Peripheral WBC count (mean \pm S.E.M.) in *S. aureus*-infected mice. Open symbols represent normal (○) and immunosuppressed (□) mice that received a 100 mg/kg oral dose of azithromycin (prophylactic model). Closed symbols represent normal (●) and immunosuppressed (■) control mice (no azithromycin). The normal baseline WBC count (mean \pm S.E.M.) is depicted in the shaded section.

than in control (non-infected) thigh tissue in both normal and leucopenic mice. Azithromycin degress from the thigh tissues of leucopenic mice was unaltered, as the elimination half-lives of leucopenic and normal mice were equivalent. The infected thigh tissue AUCs were at least two-fold greater than the AUCs from non-infected thigh tissue in normal and leucopenic mice ($P < 0.05$). The infected and non-infected thigh tissue AUCs in leucopenic mice were larger than the comparable infected and non-infected thigh tissue AUCs in normal mice; however, when the azithromycin thigh tissue concentrations in leucopenic mice are normalized for the progressive reduction in body weight of leucopenic mice (25%) relative to normal mice, the tissue C_{max} and AUC values are not significantly different.

The bacterial population rapidly reached a refractory stage and therapy with azithromycin (as well as with other macrolides) was ineffective in reducing the *S. aureus* population in the infected thigh tissues (data not shown). The bacterial strain used in this infection model is not acutely lethal to normal mice (Retsema *et al.*, 1993); however, evaluation with the strain with concomitant cytoreductive therapy has not been documented. Therefore, blood cultures were taken and a bacteraemia was observed in 60% of infected normal (3/5) and in 100% of leucopenic mice (5/5) without therapy at 2.75 days post-challenge, while bacteraemia was not observed with azithromycin therapy in either normal (0/5) or leucopenic mice (0/5).

Prophylactic mode of administration

Prophylaxis with azithromycin was evaluated by initiating drug dosing 1 h after a *S. aureus* im challenge. The immunosuppressive therapy was reduced to daily 20 mg/kg ip injections of cyclophosphamide, since severe weight loss was observed within one week in infected immunosuppressed mice in the therapeutic evaluation. The peripheral WBC counts in the prophylactic mode are displayed in Figure 3. Cyclophosphamide treatment reduced the peripheral WBC count by 70% ($P < 0.001$), induced and maintained

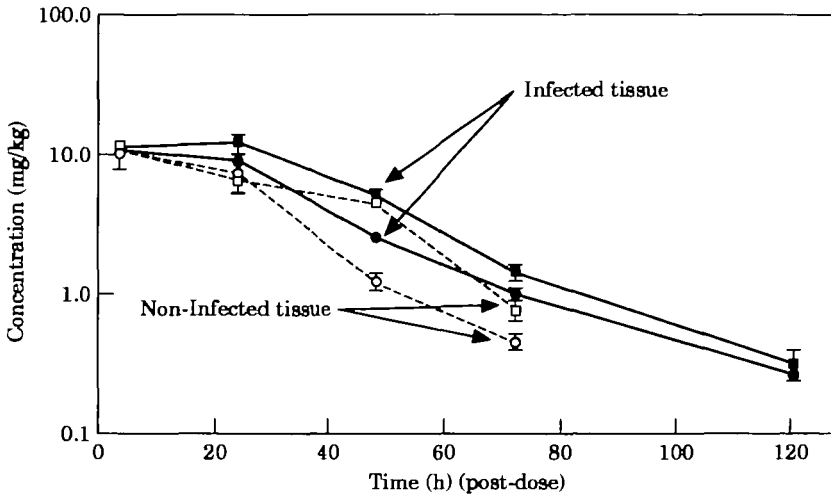


Figure 4. Azithromycin concentrations (mean \pm s.e.m.) in thigh tissues of mice following a 100 mg/kg oral dose (prophylactic model). Closed symbols represent *S. aureus*-infected thigh tissues from normal (\bullet) and immunosuppressed (\blacksquare) mice. Open symbols represent non-infected thigh tissues from normal (\circ) and immunosuppressed (\square) mice.

granulocytopenia ($<0.3 \times 10^9/L$ granulocytes) in infected azithromycin-treated mice, but was insufficient to suppress the granulocytosis evident in control (non-dosed) infected mice. Infected normal mice developed a general leucocytosis with an underlying granulocytosis ($>0.3 \times 10^9/L$ granulocytes), which was suppressed with azithromycin prophylaxis.

Azithromycin concentrations in *S. aureus*-infected and non-infected thigh tissues from normal and leucopenic mice following administration of a 100 mg/kg oral dose are presented in Figure 4. The infected and non-infected thigh tissue C_{max} values of azithromycin from leucopenic and normal mice were similar (Table II). Azithromycin elimination half-lives from infected thigh tissues were comparable from non-infected thigh tissues in both normal and leucopenic mice. The overall penetration (AUC) of azithromycin into infected thigh tissues was 36–42% greater than the corresponding AUC in non-infected thigh tissues in normal and leucopenic mice. The infected and non-infected thigh tissue AUCs in leucopenic mice are marginally larger than the comparable infected and non-infected thigh tissue AUCs in normal mice; however, when the azithromycin thigh tissue concentrations in leucopenic mice are normalized

Table II. Mean (s.e.m.) pharmacokinetic parameters of azithromycin in *S. aureus*-infected and non-infected mouse thigh tissues following administration of a 100 mg/kg oral dose given in a prophylactic mode

Parameter	Thigh tissues type			
	normal infected	normal non-infected	leucopenic infected	leucopenic non-infected
C_{max} (mg/kg)	11.5 (1.0)	10.4 (1.8)	12.7 (1.3)	12.2 (1.1)
$T_{1/2}$ (h)	20.2 (2.7)	14.2 (1.9)	17.8 (1.0)	18.3 (0.9)
$AUC_{0-\infty}$ (mg · h/kg)	460 (33)	338 (12)	619 (120)	436 (20)

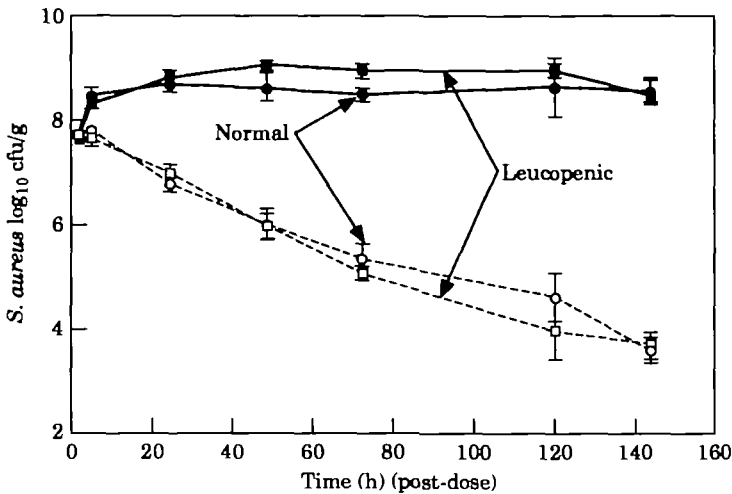


Figure 5. Recovery of *S. aureus* cfu (geometric mean \pm s.e.m.) from infected mouse thigh tissues. Open symbols represent normal (○) and immunosuppressed (□) mice that received a 100 mg/kg oral dose of azithromycin (prophylactic model). Closed symbols represent normal (●) and immunosuppressed (■) control mice (no azithromycin).

for the progressive reduction in body weight of leucopenic mice (10%) relative to normal mice, the tissue AUC values of leucopenic and normal mice are essentially equivalent.

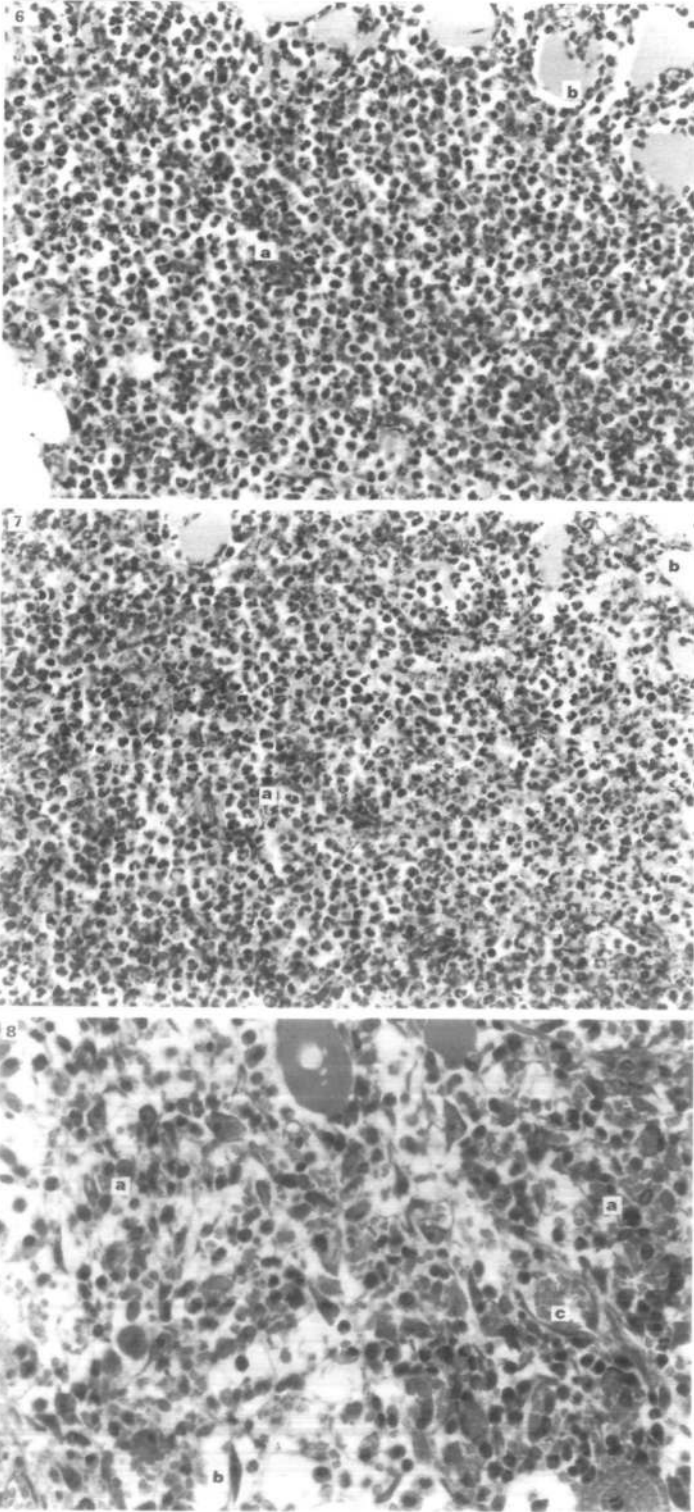
The efficacy of azithromycin prophylaxis was assessed by evaluating the reduction of *S. aureus* cfu recovered from infected thighs. The initial inoculum of 10^6 cfu/mouse increased to greater than 10^8 cfu/g by 4 h after challenge and remained $>10^8$ cfu/g through 144 h after challenge. Azithromycin prophylaxis resulted in a slow bactericidal activity (by 72 h following treatment) relative to non-dosed infected controls over a 6-day period (>4 log decrease in cfu) in both normal and leucopenic mice (Figure 5).

Histology of infected thigh

The histological changes observed in normal mice (Retsema *et al.*, 1993) were indistinguishable from those which occurred in these leucopenic mice. The *S. aureus* infection produced a focus of inflammation with early (24 h) infiltration of neutrophils and macrophages in normal (Figure 6) and leucopenic mice (Figure 7). The massive influx of phagocytic cells was accompanied by muscle cell necrosis/degeneration in both normal and granulocytopenic mice. By 72 h, there was extensive neutrophil degeneration accompanied by continued neutrophil infiltration and the onset of organization characterized by the appearance of fibroblasts and capillaries in normal and leucopenic mice (Figure 8). Additionally, the histopathology recorded for azithromycin-treated and non-treated leucopenic mice were indistinguishable as was documented for immune competent mice (Retsema *et al.*, 1993).

Discussion

Serum concentrations of antibiotics have routinely been used by clinicians as a surrogate for drug concentrations at infection sites (Moellering, 1991; Nix *et al.*, 1991). For



Figures 6–8.

erythromycin, the time that serum levels exceed the MIC is the most significant parameter determining efficacy against a variety of pathogens (Vogelman *et al.*, 1988). In this investigation, serum concentrations for azithromycin were not used as substitutes for tissue concentrations since it has been recognized that serum concentrations do not correlate with experimental and clinical efficacy (Girard, Girard & Retsema, 1990; Schentag & Ballow, 1991; Foulds & Johnson, 1993).

A phagocyte delivery mechanism for azithromycin has been proposed by Gladue *et al.* (1989). In this delivery mechanism, azithromycin is transported to the site of infection by phagocytic cells that contain high intracellular concentrations of drug. These high intracellular concentrations are attained by diffusion processes with an equilibrium which favours intracellular concentration and are enhanced by cell-to-cell interactions with fibroblasts (Gladue & Snider, 1990). These high intracellular concentrations are slowly released when extracellular concentrations of drug decrease, but are readily released during the process of phagocytosis and degranulation. This phagocyte delivery mechanism for azithromycin has been supported by infection models that correlate the association of sustained high levels of azithromycin at infection sites with phagocytic infiltration and resulting efficacy (Girard, Cimochoowski & Faiella, 1991; Retsema *et al.*, 1993).

Since a phagocyte delivery system has been suggested for azithromycin, the use of azithromycin in conditions of impaired immunity has become suspect. Apprehension arises in the treatment of the aged, alcoholic, diabetic or steroid user where subtle or serious immunocompromised conditions can exist (Drutz & Graybill, 1980). Additionally, Gram-positive infections in granulocytopenic patients are becoming more common and warrant the investigation of macrolide prophylaxis, especially in the case of penicillin allergy (Brown, 1984).

The cytoreductive agent, cyclophosphamide, was used in our investigation to induce a leucopenia with underlying granulocytopenia (Calame, Guiot & Mattie, 1990) and the degree and maintenance of granulocytopenia were documented. Leucocytosis was observed in normal infected mice, while leucopenia was induced and maintained in cyclophosphamide-treated mice. Azithromycin administration resulted in a reduction in the peripheral WBC count relative to non-treated infected mice, which suggests that reduction in the bacterial population by azithromycin minimized the recruitment of granulocytes.

The penetration of antibodies into infection foci has long been a matter of interest and controversy (Eagle, 1952; Joiner *et al.*, 1981; Hoogeterp *et al.*, 1987). For most antibiotics, lower levels of drug are found in infection foci as a result of decreased penetration and increased degradation of drug (Joiner *et al.*, 1981). In contrast, augmentation of penetration and residence in infection sites is observed with azithromycin (Girard *et al.*, 1990, 1991; Retsema *et al.*, 1993).

Figure 6. Early focus of inflammation (24 h) following *S. aureus* infection in normal mice; showing infiltration of (a) neutrophils and macrophages, as well as accompanying (b) muscle cell necrosis/degeneration (magnification $\times 625$).

Figure 7. Early inflammation focus (24 h) in leucopenic mice; showing infiltration of (a) neutrophils and macrophages, as well as accompanying (b) muscle cell necrosis/degeneration (magnification $\times 625$).

Figure 8. Inflammation focus (72 h) in normal mice; showing (a) neutrophil infiltration and degeneration, and evidence of the onset of organization by the appearance of (b) fibroblasts and (c) capillaries (magnification $\times 625$).

Leucopenic mice have fewer circulating granulocytes than normal mice; therefore, infection sites in leucopenic mice should contain lower concentrations of azithromycin, relative to normal mice, if delivery of drug is entirely under control of a phagocytic delivery system. On the contrary, a moderate increase in the penetration of azithromycin was detected in the infected tissues of leucopenic mice; although, when the concentrations in leucopenic mice are normalized for the progressive reductions in body weight, the penetration of azithromycin in leucopenic and normal mice is indistinguishable. Since, under these experimental conditions, the elimination half-life of azithromycin was equivalent in normal and leucopenic mice, cyclophosphamide therapy has no impact on the elimination of azithromycin. This apparent paradox in drug delivery in leucopenic mice can be partially explained by the fact that massive infiltration of granulocytes into the infection sites occurred throughout the observation period, despite the reduction in granulocyte numbers in peripheral blood. This granulocyte recruitment suggests that, once outside the peripheral blood, granulocytes are less sensitive to cyclophosphamide cytoreductive activity or, since bone marrow suppression was not observed, immature granulocytes were recruited.

Comparison of the therapeutic and prophylactic modes of azithromycin administration in normal and granulocytopenic mice indicates substantial differences in elimination half-lives from infected versus non-infected tissues. The half-life of elimination from infected tissues was ~three-fold longer in the therapeutic model relative to the prophylactic model of normal and leucopenic mice. The more rapid degree of azithromycin in the prophylactic model is probably a result of reduction in the formation of penetration barriers as a consequence of a reduction in the bacterial population, which was brought about by prophylaxis with azithromycin.

Although the concentration versus time profiles and respective AUCs for azithromycin in leucopenic mice are somewhat greater than the comparable values in normal mice, if these values are normalized for the progressive reductions in the mean body weight of leucopenic mice relative to normal mice over the evaluated time interval, then the observed pharmacodynamics of azithromycin are equivalent in normal and leucopenic mice in both of these models. In contrast, decreases in azithromycin exposures have been reported in pneumococcal infected pulmonary tissues of immunosuppressed mice relative to immune competent mice (Veber *et al.*, 1993). This contrast could be the result of the many differences between the two models; the immunosuppression in the pulmonary infection was more prolonged and severe than the moderate immunosuppression in the thigh infection model and likely resulted in a greater reduction in the number of whole body leucocytes. The pulmonary tissues are more highly perfused with blood than are muscle tissues, therefore the potential contribution in local concentration by phagocytic cells is greater in a pulmonary infection, and in pulmonary tissues alveolar macrophages that efficiently concentrate azithromycin are a resident cell type even without infection (Baldwin *et al.*, 1990; Honeybourne *et al.*, 1990), while tissue macrophages are rare in uninfected muscle tissue and are not a predominant cell type even in the infected muscle. The sustained high concentrations of azithromycin in the infected tissues resulted in suppression of bacteraemia in the therapeutic mode as well as bactericidal activity in the prophylactic mode. This slow bactericidal activity has been reported in other *S. aureus* models of localized infection (Retsema *et al.*, 1990; Girard *et al.*, 1991) and is distinctive for azithromycin, as macrolide antibiotics usually display a bacteriostatic character (Calame *et al.*, 1990). In a *Haemophilus influenzae* pulmonary infection model, Vallée

et al. (1992) have reported a slow clearance of *H. influenzae* by azithromycin (2.55 log cfu reduction in 36 h) with a more rapid bactericidal activity following two doses (4.93 log cfu reduction in 36 h) and Azoulay-Dupuis *et al.* (1991) have reported a rapid clearance of *S. pneumoniae* by azithromycin in a chronic pneumococcal pulmonary infection model (>4 log cfu reduction in 17 h). Consequently, depending on the bacterial pathogen, the site of infection and the dosing regimen, variable (rapid and slow) clearance rates for azithromycin have been reported. The lack of bactericidal activity in the therapeutic model is readily explained by the inappropriate timing of dose, whereby, the dose was administered when the bacterial population had reached a stationary phase of growth and was refractory to antibiotic intervention. This refractory condition has been reported even with bactericidal penicillins and has been attributed to a dormant stage (occurring during stationary phase where bacterial metabolism is minimal due to depletion of available nutrients) as well as to the presence of "persisters" within the bacterial population (Eagle, 1952).

This study indicates that the pharmacokinetics and pharmacodynamics of azithromycin in infected tissues are unimpaired in leucopenic mice. Furthermore, since azithromycin concentrations in infected tissues were consistently higher than normal tissue concentrations, serum levels have been shown to be inadequate to assess tissue concentrations and predict clinical efficacy of azithromycin. These high sustained concentrations of azithromycin in *S. aureus*-infected thigh tissues in leucopenic mice resulted in a slow bactericidal activity which was indistinguishable from that seen in normal mice, and histological examination suggests that the leucopenia with underlying granulocytopenia observed in the peripheral blood does not necessarily reflect the phagocytic status at the site of infection. In summary, this experimental evidence suggests that azithromycin's clinical usefulness should not necessarily be precluded for treatment of infections, for which it is indicated, should moderate immunosuppression be suspected.

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